Western Blot Analysis: Protein Quantification

Section of Cancer Genomics, Genetics Branch, NCI
National Institutes of Health

Reagents

BCA Protein Assay Reagent Kit
Pierce, Cat. 23225

Preparation

Lysis Buffer + Protease Inhibitors (PIH)
See "Protein Extracts for Westerns"
BSA Standards
Make standards by diluting supplied BSA [2 mg/ml] into appropriate Lysis Buffer + PIH as in the following chart:

Tube Name	Volume of BSA	From Tube	Volume Lysis Buffer	Final [BSA]
	30.0 µl of	STOCK	0 µl	2.00 mg/ml
A	37.5 µl of	STOCK	12.5 µl	1.50 mg/ml
B	32.5 µl of	STOCK	32.5 µl	1.00 mg/ml
C	17.5 µl of	A	17.5 µl	0.75 mg/ml
D	32.5 µl of	B	32.5 µl	0.50 mg/ml
E	32.5 µl of	D	32.5 µl	0.25 mg/ml
F	32.5 µl of	E	32.5 µl	0.125 mg/ml
G	10.0 µl of	F	40.0 µl	0.05 mg/ml

Procedure

1. Thaw extracts on ice.

2. Use Pierce BCA kit, following directions inside.

3. Make 1:10 dilutions of each cell lysate into Lysis Buffer + PIH and from these mix 1µl diluent into 4 µl Lysis Buffer + PIH for a final dilution of 1:50.

4. Make working solution (WS) by combining 2500 µl Soln. A + 50 µl Soln. B from BCA Protein Assay Reagent Kit.

5. Mix 5 µl each standard and 5 µl each 1:50 dilution with 95 µl working solution for a final dilution of 1:20.

6. Incubate at 37˚C for 30 min.

7. Place tubes in ice/water slurry to inhibit reaction while reading absorption at 562nm.